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Seasonal assessment of environmental tobacco smoke and respirable suspended particle exposures for nonsmokers in Bremen using personal monitoring

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Abstract

The study was designed to determine seasonal differences in personal exposures to respirable suspended particles (RSP) and environmental tobacco smoke (ETS) for nonsmokers in Bremen, Germany. The subjects were office workers, either living and working in smoking locations or living and working in nonsmoking locations. One hundred and twenty four randomly selected nonsmoking subjects collected air samples close to their breathing zone by wearing personal monitors for 24 h or, in some cases, for 7-day periods during the winter of 1999. The investigation was repeated in the summer with 126 subjects, comprised of as many of the studied winter population (89 subjects) as possible. Saliva cotinine analyses were undertaken to verify the nonsmoking status of the subjects. Subjects wore one personal monitor while at work and one while away from the workplace on weekdays, and a third monitor at the weekend. Collected air samples were analysed for RSP, nicotine, 3-ethenylpyridine (3-EP) and ETS particles. The latter were estimated using ultraviolet absorbance (UVPM), fluorescence (FPM) and solanesol (SolPM) measurements. ETS exposure was consistently higher in the winter than in the summer, this pattern being particularly evident for subjects both living and working with smokers. The highest median 24-h time weighted average (TWA) concentrations of ETS particles (SolPM, 25 $\mu g\ m^{-3}$) and nicotine (1.3 $\mu g\ m^{-3}$) were recorded for subjects performing weekday monitoring during the winter. These were significantly higher than equivalent levels of ETS particles (SolPM, 2.4 $\mu g\ m^{-3}$) and nicotine (0.26 $\mu g\ m^{-3}$) determined during the summer. There were no appreciable differences between winter and summer percent workplace contributions to median TWA ETS particle and nicotine weekday concentrations, the workplace in Bremen, in general, contributing between 35% and 61% of reported median concentrations. Workers, on average, spent one-third of their time at work during a weekday, indicating that concentrations were either comparable or higher in the workplace than in the home and other locations outside the workplace. Median 24-h weekend ETS particle and nicotine concentrations for smoking locations were not significantly different from equivalent weekday levels during the winter, but were significantly lower during the summer. Based upon median 24-h TWA SolPM and nicotine concentrations for the winter, extrapolated to 1 year's ETS exposure, those subjects both living and working in smoking locations (the most highly exposed group) would potentially inhale 13 cigarette equivalents/year (CEs/y). However, based on a similar extrapolation of summer measurements, the same group of subjects would potentially inhale between 1.3 and 1.9 CEs/y. The most highly exposed subjects in this study, based upon 90th percentile concentrations for those both living and working in smoking locations during the winter, would potentially inhale up to 67 CEs/y in the winter and up to 22 CEs/y in the summer. This clearly demonstrates that seasonal effects should be taken into account in the design and interpretation of ETS exposure studies. Air sampling over a 7-day period was shown to be technically feasible, and subsequent RSP, ETS particle and nicotine levels determined by 7-day monitoring were not found to be significantly different from equivalent levels determined by 24-h monitoring. However, the longer sampling period resulted in the collection of an increased quantity of analytes, which improved the limits of quantitation (LOQ) and allowed a more accurate determination of low level ETS exposure. This was reflected by a reduced percentage of data falling below the LOQ for 7-day monitoring compared with 24-h monitoring. The use of a liquid chromatographic method with tandem mass spectrometric detection for saliva cotinine measurement afforded a greatly improved LOQ and greater accuracy at low concentrations compared with the radioimmunoassay (RIA) method used in previous studies by these authors. In this study, 17 subjects out of 180 tested (9.4%) were found to have saliva cotinine levels exceeding the selected threshold of 25 $ng\ ml^{-1}$ used to discriminate between smokers and nonsmokers.

Keywords: Personal exposure; Environmental tobacco smoke; Respirable suspended particles; Nicotine; Cotinine

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1. Introduction

During the 2-year period from November 1994 to November 1996, these authors have performed air quality investigations in Stockholm (Phillips et al., 1996), Barcelona (Phillips et al., 1997a), Turin (Phillips et al., 1997b), Paris (Phillips et al., 1998a), Bremen (Phillips et al., 1998b), Lisbon (Phillips et al., 1998c), Basel (Phillips et al., 1999), Prague (Phillips et al., 1998d), Hong Kong (Phillips et al., 1998e), Kuala Lumpur (Phillips et al., 1998f), Sydney (Phillips et al., 1998g) and Beijing (Phillips et al., 1998h). The aim of these investigations was to determine the personal exposures to respirable suspended particles (RSP) and environmental tobacco smoke (ETS) particles for nonsmoking inhabitants of each city by obtaining accurate measurements of air concentrations. This was achieved by studying two groups of nonsmokers in each city, with the exception of Sydney, where a slightly modified protocol was followed. One group consisted of housewives who wore a monitor for 24 h, and a second group consisted of office workers who wore one monitor while at work and a separate monitor while away from the workplace, again for a total of 24 h. These groups were further subdivided into six 'cells' for investigation according to whether the home or workplace was designated as smoking or nonsmoking.

These studies have provided valuable information regarding personal exposures to RSP and ETS in a variety of situations and have given insight regarding cultural and legislative differences between the geographical locations investigated. As a result of the experiences accumulated during the course of these studies, a number of opportunities to improve study design have been considered. Of particular note was the proportion of ETS marker concentration data falling below the limit of quantitation. This was particularly apparent for cells with low exposures and short sampling times, and for geographical locations where overall levels of ETS exposure were low. Also highlighted as an area for improvement was the limit of quantitation (1 ng ml^{-1}) achieved for the determination of saliva cotinine concentrations using a radioimmunoassay (RIA) method. Many subjects have saliva cotinine concentrations below this limit. Although saliva cotinine information was originally only intended for use as a means for determining smoker misclassification, there has been an indication throughout our investigations that this measure may be suitable for use as a biomarker for ETS exposure, particularly for group comparisons. However, the assay was not sufficiently sensitive to show any significance of differences between cells where measured ETS concentrations were low, particularly apparent in geographical locations where overall levels of ETS were minimal.

In these authors' previous studies, annual estimates for ETS exposure have assumed that weekend exposures were equivalent to weekday exposures outside the workplace. Any potential differences between weekday and weekend measurements have yet to be fully investigated. The question as to whether a 24-h collection period was of sufficient length to be truly representative of an individual's exposure, or whether longer periods of study should be considered was also unanswered. The study reported in this publication has incorporated measures to improve upon these highlighted areas, in addition to other design modifications.

However, the main objective of this study was to determine what influence the season of the year would have upon determined RSP and ETS concentrations by comparison of personal exposures for nonsmoking workers in Bremen during the winter and summer. Office workers were recruited into two cells; those living and working in nonsmoking environments in one cell, and those living and working in smoking environments in the other. These were the cells where lowest and highest RSP and ETS concentrations had been found in previous studies and were selected to provide information regarding the lowest and highest limits of exposure scenarios. Each subject wore one personal monitor while at work and a separate monitor for all locations outside the workplace over a 24-h period. In addition to carrying out air monitoring during the working week, subjects also undertook a second 24-h monitoring period wearing a third personal monitor, on 1 day at the weekend. In this way, a more accurate assessment of annual exposure could be made, compared with these authors' previous studies in which the 'home' portion of weekday measurements was extrapolated to encompass weekend exposure.

In parallel with 24-h monitoring, additional subjects were recruited to monitor exposures over an entire week, thereby enabling a direct comparison of 'snapshot' monitoring vs. a more extended collection period. As with 24-h monitoring, this was achieved using three personal monitors, one for use at work during the week, one for use away from the workplace during the week and the third for use over the whole weekend. As highlighted in a recent review of occupational exposure to ETS and health risk assessment (Jaakola and Samet, 1999), consideration must be given to the most appropriate methods for ETS exposure assessment. This was these authors' first attempt at 7-day sampling, the intention being to investigate whether such sampling was feasible, and whether more representative exposure measurements and better limits of quantitation (LOQ) for low exposure levels could be achieved.

The winter portion of the study was conducted during February/March 1999 and the summer portion during June/July 1999. As in previous studies, personal air monitoring was chosen for this study in preference to static or ambient measurements in order to represent personal exposures to selected pollutants as accurately as possible. ETS particles were estimated using ultraviolet absorbing particulate matter (UVPM), fluorescing particulate matter (FPM) and solanesol related particulate matter (SolPM). Vapour phase ETS exposures were also assessed by simultaneous measurement of nicotine and 3-ethenylpyridine (3-EP) concentrations. Subjects also provided saliva samples for cotinine analysis

and recorded their activities and observations using diaries and questionnaires. Similar methodologies have been used in other recent studies (Sterling et al., 1996; Jenkins et al., 1996; Baek et al., 1997).

2. Methods

2.1. Recruitment of subjects

Recruitment was performed by Trend Research, a market research bureau based in Hamburg, Germany. A random sample population was selected from their database to be compliant with the following criteria wherever possible:

1. All subjects to be employed nonsmokers living within 15 km of Bremen's city centre.
2. Equal proportions from three age groups 20–34, 35–49 and 50–65.
3. Subjects' lifestyles to closely resemble the population within 15 km of the city centre.
4. Subjects to be distributed between two 'cells' as indicated in Table 1.

Subjects were recruited by telephone using numbers selected at random from a database of potential volunteers, who were screened to confirm their eligibility to participate in the study. Suitable volunteers were given an appointment to attend an information/training session organised at the Hotel Mercure Columbus in Bremen. In order to assign subjects into each cell, as depicted in Table 1, homes were classified as "smoking" if a smoker of cigarettes, pipes or cigars was resident and also normally smoked within communal areas of the household. The smoking status of a workplace was defined by the absence/presence of smoking coworkers within 30 m of the subject's workstation.

It was intended that subjects were recruited for participation in both winter and summer investigations, although it was expected that a certain proportion would be unwilling or unable to participate in the summer investigation, following their participation during the winter. In order to compensate for this expected outfall, and thereby minimise the difference between subject populations investigated in winter and summer, a 20% overage of required subject numbers was recruited for the winter investigation. Additional subjects were recruited for the summer investigation in order to achieve required numbers for participation.

Table 1
Cell categorisation by home and workplace status (Bremen)

	Smoking status		Planned number	
Cell	Household	Workplace	24 h	7 days
Winter investigation				
1	Smoking	Smoking	60	12
2	Nonsmoking	Nonsmoking	60	12
Summer investigation				
1	Smoking	Smoking	50	10
2	Nonsmoking	Nonsmoking	50	10

2.2. The monitoring session

Subjects were required to wear a personal monitor designed to collect particulate and vapour phase components in the air close to their breathing zone. The sampling system used was the same as that reported by Ogden et al. (1996), with the exception that black conductive filter cassettes (A-003750-AC, Omega Specialty Instrument, Chelmsford, MA, USA), without gaskets, were used in place of transparent polystyrene units (M00003700, Millipore UK, Herts, England). RSP and ETS particles were collected onto a Fluoropore membrane filter, nicotine and 3-EP were adsorbed onto XAD-4 resin beads. Data generated by personal monitors found to be out of calibration on their return (flow rates varying by more than 10%), possibly resulting from tampering, misuse or equipment malfunction were excluded. The personal monitoring methodology has been described in detail elsewhere (Phillips et al., 1996) and consisted briefly of the following.

- *Initial visit to the study centre:* On arrival, subjects were shown an instructional video, dubbed into German, explaining the objectives of the air quality survey and locally recruited interpreters gave further instructions regarding use of the equipment and how to complete the documentation. Subjects were issued with German language questionnaires and diaries for recording exposures and observations over the 24-h or 7-day collection periods and were supervised during completion of a "first visit" questionnaire. In order to avoid misinterpretation and possible errors in translation, all questionnaires and diaries were designed for either numeric or tick-box answers. Subjects were provided with two personal monitors for use during their working weekday(s) along with diaries and questionnaires for recording observations related to air quality throughout the monitoring period. Subjects were required to provide a saliva sample prior to the monitoring period (presample).
- *Interim visit to the study centre:* Following completion of the weekday monitoring period (24 h or 5 days), subjects returned their personal monitors and associated documentation to the study centre and collected their personal monitor, diaries and questionnaires for the weekend monitoring period. At this point subjects were required to provide a second saliva sample (interim sample). Subjects performing 7-day monitoring wore their weekday monitors from Monday morning until their return to the study centre on Friday evening. The weekend monitor was then worn from this time until the following Monday morning.

• *Final visit to the study centre:* Following completion of the weekend monitoring period, subjects returned their personal monitors and associated documentation to the study centre. Subjects also provided a third and final saliva sample (postsample) and completed a "last visit" questionnaire.

For this investigation, professional battery packs (All-Batteries (UK), Herts, UK) were used in preference to battery holders for housing disposable batteries. Each disposable battery pack comprised 4 × D cell alkaline batteries, with an integral fly lead and connector suitable for direct connection to the pump. In previous studies conducted by these authors, air sampling pumps were powered by 4 × C cell alkaline batteries, which were suitable for continuous pump operation for up to 35 h. The use of D size batteries in this study enabled continuous pump operation for up to 70 h, which was sufficient to cover the required weekend sampling period (approximately 60 h) for subjects performing 7-day monitoring. However, these subjects were issued with a spare battery pack for replacement of the unit within their weekday home monitor, which had the potential to be operated for longer than 70 h in total. Subjects recruited for 7-day monitoring were provided with training for the replacement of battery packs, and were asked to replace the battery pack in their home monitor after the second or third day of sampling. Replacement battery packs were uniquely identified in order to confirm that subjects had carried out the required replacement procedure.

3. Analytical procedures

All analytical procedures were validated and have been fully described previously by these authors (Phillips et al., 1996). In this study, the following analytes were determined.

(1) RSP — using a gravimetric procedure (Ogden et al., 1990).

(2) Saliva cotinine and 3-hydroxycotinine — using a high-performance liquid chromatography procedure with tandem mass spectrometric detection (LC–MS/MS) (Bentley et al., 1999).

(3) Nicotine and 3-EP — using a capillary gas chromatography (GC) procedure with nitrogen specific detection (Ogden et al., 1989).

(4) Estimation of ETS particles (three procedures) — using high-performance liquid chromatography (HPLC) procedures to determine the ultraviolet absorbance (UVPM), fluorescence (FPM) or solanesol content (SolPM) of methanolic filter extracts (Ogden et al., 1990, Phillips et al., 1996). The factors used in this study to convert instrument responses into an equivalent concentration of ETS particles were 41 (SolPM), 43 (FPM) and 7.8 (UVPM) as determined by Nelson et al. (1997) for cigarettes on the German market.

The analytical LOQ for these analyses are presented in Tables 2 and 3, for 24-h and 7-day monitoring, respectively, together with the proportion of data below the LOQ. In order to calculate summary statistics, any data below the analytical LOQ were assigned a value of ½LOQ, rather than zero, prior to the calculation of exposure concentration using the corresponding air sampling volume. The LOQs expressed as air concentrations in these tables are therefore only an approximation as they varied for each sample dependent upon the sampling pump flow rate and monitoring time.

4. Subject selection

Of the 139 subjects that were initially recruited for the winter portion of the study, one subject did not make any sample collections due to illness and a further 14 subjects were excluded because their saliva cotinine levels were above the selected threshold (25 ng ml^{-1}) for nonsmokers.

Table 2
LOQ for the analytical methods and corresponding air concentrations according to 24-h monitoring collection period (Bremen)

		LOQ expressed as an air concentration according to sampling time (μg m^{-3})[a]			Proportion of data below the LOQ	
Measurement	Analytical LOQ	23.4 h[b]	15.3 h[c]	8.5 h[d]	Winter (%)	Summer (%)
RSP	13 μg/filter	5.4	8.2	14.8	5	7
ETS particles measured by UV (UVPM)	1.17 μg/filter	0.48	0.74	1.33	8	13
ETS particles measured by fluorescence (FPM)	0.27 μg/filter	0.11	0.17	0.31	0	5
ETS particles measured by solanesol (SolPM)	0.62 μg/filter	0.26	0.39	0.71	37	68
Nicotine	0.05 μg/tube	0.071	0.11	0.20	47	64
3-EP	0.05 μg/tube	0.071	0.11	0.20	46	59
Saliva cotinine	0.05 ng ml^{-1}	–	–	–	4	4
Saliva 3-hydroxycotinine	0.10 ng ml^{-1}	–	–	–	31	48

[a] A flow rate of 1.72 l min^{-1} through the Fluoropore filter was assumed in the LOQ calculation for RSP, UVPM, FPM and SolPM. The LOQ calculation for nicotine and 3-EP assumed a flow rate of 0.50 l min^{-1} through the XAD-4 tube.
[b] Mean sampling time at the weekend for subjects in Bremen (24-h monitoring).
[c] Mean sampling time outside the workplace for subjects in Bremen (24-h monitoring).
[d] Mean time spent at work for subjects in Bremen (24-h monitoring).

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Table 3
LOQ for the analytical methods and corresponding air concentrations according to 7-day monitoring collection period (Bremen)

Measurement	Analytical LOQ	LOQ expressed as an air concentration according to sampling time ($\mu g\ m^{-3}$)[a]			Proportion of data below the LOQ	
		63.0 h[b]	60.6 h[c]	39.3 h[d]	Winter (%)	Summer (%)
RSP	13 μg/filter	2.0	2.1	3.2	0	5
ETS particles measured by UV (UVPM)	1.17 μg/filter	0.18	0.19	0.29	0	5
ETS particles measured by fluorescence (FPM)	0.27 μg/filter	0.042	0.043	0.067	0	3
ETS particles measured by solanesol (SolPM)	0.62 μg/filter	0.095	0.099	0.15	17	67
Nicotine	0.05 μg/tube	0.026	0.028	0.042	20	32
3-EP	0.05 μg/tube	0.026	0.028	0.042	15	14

[a] A flow rate of 1.72 l min^{-1} through the Fluoropore filter was assumed in the LOQ calculation for RSP, UVPM, FPM and SolPM. The LOQ calculation for nicotine and 3-EP assumed a flow rate of 0.50 l min^{-1} through the XAD-4 tube.

[b] Mean sampling time outside the workplace for subjects in Bremen (7-day monitoring).

[c] Mean sampling time at the weekend for subjects in Bremen (7-day monitoring).

[d] Mean time spent at work for subjects in Bremen (7-day monitoring).

Of the remaining 124 subjects who successfully completed the winter portion of the study, 89 went on to participate in the summer (representing 72% of the initial population) and additional subjects (41) were recruited to make up the required total. Of the 130 subjects who participated in the summer, one subject was excluded having admitted to smoking during the study period, and a further three subjects were excluded because their saliva cotinine levels were above the selected threshold (25 ng ml^{-1}) for nonsmokers. In total, 126 subjects successfully completed the summer portion of the study.

The age and gender distributions of the subjects who successfully completed the study are presented in Table 4. The gender distribution was very close to the planned 50% per gender for both winter (50% male, 50% female) and summer (52% male, 48% female) portions of the study, being close to the actual gender distribution for office workers in Bremen (55% male, 45% female). The age distribution for office workers showed a considerable variation from the planned 33% for each age group. Approximately 41% of the recruits fell into each of the younger age groups (20–34 and 35–49 years) and the remaining 18% were aged between 50 and 64 years for both winter and summer investigations. This was, however, consistent with the actual age spread for office workers in Bremen comprising 40% aged 20–34 years, 38% aged 35–49 years and 18% aged 50–64 years.

Table 4
Age and gender distribution for study subjects (Bremen)

Cell[a]	Monitoring period	Gender		Age range			Total
		Males	Females	20–34	35–49	50–64	
Winter investigation							
1	24 h	23	26	23	22	4	49
	7 day	3	6	2	5	2	9
2	24 h	30	23	21	20	12	53
	7 day	6	7	4	4	5	13
Summer investigation							
1	24 h	28	24	23	24	5	52
	7 day	6	6	4	6	2	12
2	24 h	27	23	23	21	6	50
	7 day	5	7	4	4	4	12
Winter total		62	62	50	51	23	124
Summer total		66	60	54	55	17	126

[a] Cell 1: smoking household/smoking workplace. Cell 2: nonsmoking household/nonsmoking workplace.

5. Weather and pollutant information

Information regarding ambient air quality during the course of the winter and summer study periods was obtained locally (Senator für Umweltschutz und Stadtentwicklung) and concentrations of particulates, NO, NO_2, SO_2, O_3 and CO were provided as daily means from a monitoring station located in the centre of Bremen. Overall mean NO_2 concentrations were low, with values of 33 and 19 $\mu g\ m^{-3}$ reported for the winter and summer study periods, respectively (ranges 19–55 and 5–34 $\mu g\ m^{-3}$). SO_2 concentrations were also low with mean concentrations of 4.9 $\mu g\ m^{-3}$ (range 3–12 $\mu g\ m^{-3}$) and 4.7 $\mu g\ m^{-3}$ (range 2–7 $\mu g\ m^{-3}$) reported for winter and summer, respectively. Based upon the UK Department of the Environment pollutant bands, these NO_2 and SO_2 concentrations indicate that the air quality in Bremen could be described as 'very good' for both the winter and summer study periods. Particulate concentrations were also relatively low with mean concentrations of 19 $\mu g\ m^{-3}$ (range 7–52 $\mu g\ m^{-3}$) and 23 $\mu g\ m^{-3}$ (range 16–46 $\mu g\ m^{-3}$) apparent over the winter and summer study periods, respectively. These concentrations were directly comparable with respective mean indoor RSP concentrations determined for nonsmoking locations. Mean temperature and relative humidity values of 4.5°C (range −3.2–9.2°C) and 78.6% (range 59.2–93.1%) RH were recorded during the winter study period and values of 17.1°C (range 14.0–20.0°C) and 59.5% (range 45.0–

Table 5
Summary statistics for TWA particle and vapour phase concentrations determined for subjects performing 24-h sampling in smoking locations (Bremen)

Analyte	Collection period[a]	Number of subjects	10th Percentile	90th Percentile	Arithmetic mean	Geometric mean	Median
RSP ($\mu g\ m^{-3}$)	Weekday winter	47	29	115	65	54	53
	Weekend winter	49	24	179	78	57	48
	Weekday summer	48	15	74	38	31	30
	Weekend summer	50	12	39	28	22	22
	Weekday 1995	17	24	86	48	42	39
SolPM ($\mu g\ m^{-3}$)	Weekday winter	47	2.1	106	40	17	25
	Weekend winter	49	0.12	124	45	9.5	20
	Weekday summer	48	0.21	42	11	2.2	2.4
	Weekend summer	50	0.12	5.6	4.4	0.38	0.12
	Weekday 1995	17	0.25	33	11	3.5	6.5
FPM ($\mu g\ m^{-3}$)	Weekday winter	47	4.0	83	37	20	23
	Weekend winter	49	2.2	119	43	18	17
	Weekday summer	48	0.53	47	15	5.0	5.4
	Weekend summer	50	0.35	18	7.6	1.7	0.93
	Weekday 1995	17	1.8	36	14	6.3	6.3
UVPM ($\mu g\ m^{-3}$)	Weekday winter	47	4.6	90	39	21	25
	Weekend winter	49	2.7	113	44	19	20
	Weekday summer	48	1.4	49	16	7.1	6.7
	Weekend summer	50	0.94	20	8.2	3.0	1.8
	Weekday 1995	14	3.4	41	18	12	9.7
Nicotine ($\mu g\ m^{-3}$)	Weekday winter	48	0.22	5.0	2.8	1.2	1.3
	Weekend winter	48	0.03	6.0	2.3	0.67	0.80
	Weekday summer	44	0.06	4.1	1.2	0.37	0.26
	Weekend summer	40	0.03	0.98	0.73	0.11	0.03
	Weekday 1995	17	0.13	2.1	1.2	0.66	0.69
3-EP[b] ($\mu g\ m^{-3}$)	Weekday winter	48	0.14	1.7	0.93	0.58	0.63
	Weekend winter	48	0.06	2.4	0.87	0.41	0.53
	Weekday summer	44	0.06	1.5	0.54	0.28	0.29
	Weekend summer	40	0.03	0.62	0.34	0.13	0.11
	Weekday 1995	17	0.14	1.3	0.69	0.48	0.61

TWA exposure concentrations, determined for each subject from measured levels both inside and outside the workplace, were used to calculate the above statistical parameters for weekday measurements.

[a] 1995 values pertain to the original Bremen study performed by these authors during May 1995.

[b] 3-EP: 3-ethenylpyridine.

73.0%) RH were recorded during the summer study period in Bremen.

6. Smoking status

Saliva cotinine levels were determined in order to verify that recruited subjects had correctly reported themselves as nonsmokers. Various threshold levels, above which subjects would be classified as smokers, have been suggested and include 10 ng ml^{-1} (Etzel, 1990), 15 ng ml^{-1} (McNeill et al., 1987), 30 ng ml^{-1} (Lee, 1987) and more recently 100 ng ml^{-1} (Sterling et al., 1996). In this study, 25 ng ml^{-1} (maximum of pre-, interim and postlevels) was chosen as a suitable cut-off level, as used and described previously by the principal author (Phillips et al., 1994). Using this threshold, 14 subjects from the winter and a further 4 subjects from the summer investigations, with levels between 26.6 and 444 ng ml^{-1} were assumed to be smokers and were excluded from the study.

In this study, subjects were required to confirm they had been nonsmokers for more than 6 months and no attempt was made to differentiate between "non" and "never" smokers. Various criteria can be used to assess the rate at which recruited subjects misreport their smoking status, including responses to questionnaires. Depending upon the criteria used, the rate at which subjects misclassified their nonsmoking status in this study ranged between 9.4% (17 from 180) and 10.1% (14 from 139). This misclassification rate falls slightly higher than the overall average rates (between 5.0% and 7.0%) observed in the European cities studied previously by these authors. A misclassification rate of 2.5% was apparent for the initial study performed in Bremen during 1995.

Etzel's (1990) review of the use of saliva cotinine for this purpose suggests that subjects with cotinine levels between 10 and 100 ng ml^{-1} may be classified as infrequent smokers, and had 10 ng ml^{-1} been selected as the cut-off level a further two subjects would have been rejected. An exclusion rate of 4.2%, using a 15 ng ml^{-1} saliva discrimination level, was also found on a recent personal air monitoring study (Jenkins et al., 1996) of 1564 subjects in the United States. A discrimination level of 15 ng ml^{-1} was also chosen in a recent follow-up population study on smoking misclassification in southern Germany (Heller et al., 1998). In this German study, the proportion of self-

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Table 6
Summary statistics for TWA particle and vapour phase concentrations determined for subjects performing 24-h sampling in nonsmoking locations (Bremen)

Analyte	Collection period[a]	Number of subjects	10th Percentile	90th Percentile	Arithmetic mean	Geometric mean	Median
RSP ($\mu g\ m^{-3}$)	Weekday winter	49	11	50	30	26	28
	Weekend winter	53	15	38	28	25	23
	Weekday summer	48	12	32	21	20	20
	Weekend summer	48	12	27	20	18	17
	Weekday 1995	33	12	38	25	23	23
SolPM ($\mu g\ m^{-3}$)	Weekday winter	49	0.22	12	4.9	1.7	1.9
	Weekend winter	53	0.12	2.2	3.9	0.30	0.12
	Weekday summer	48	0.20	1.9	0.75	0.40	0.21
	Weekend summer	48	0.12	0.12	0.32	0.15	0.12
	Weekday 1995	34	0.24	0.87	0.89	0.39	0.26
FPM ($\mu g\ m^{-3}$)	Weekday winter	49	0.85	14	5.9	3.5	3.0
	Weekend winter	53	0.82	4.3	5.3	1.9	1.6
	Weekday summer	48	0.29	4.9	1.9	1.0	0.91
	Weekend summer	48	0.20	3.7	1.4	0.56	0.41
	Weekday 1995	34	0.38	3.2	1.5	0.96	0.65
UVPM ($\mu g\ m^{-3}$)	Weekday winter	49	0.96	15	6.4	3.8	3.5
	Weekend winter	53	1.1	4.9	5.8	2.3	1.8
	Weekday summer	48	0.66	6.1	2.7	1.8	1.7
	Weekend summer	48	0.51	4.0	2.2	1.3	1.1
	Weekday 1995	31	1.3	4.5	2.8	2.2	1.7
Nicotine ($\mu g\ m^{-3}$)	Weekday winter	50	0.06	0.99	0.42	0.16	0.12
	Weekend winter	53	0.03	0.19	0.20	0.05	0.03
	Weekday summer	43	0.05	0.26	0.13	0.08	0.06
	Weekend summer	41	0.03	0.10	0.07	0.04	0.03
	Weekday 1995	35	0.08	0.24	0.16	0.13	0.10
3-EP[b] ($\mu g\ m^{-3}$)	Weekday winter	50	0.06	0.49	0.21	0.13	0.09
	Weekend winter	53	0.03	0.28	0.13	0.05	0.03
	Weekday summer	43	0.06	0.25	0.12	0.09	0.06
	Weekend summer	41	0.03	0.17	0.07	0.05	0.03
	Weekday 1995	35	0.08	0.25	0.13	0.11	0.09

TWA exposure concentrations, determined for each subject from measured levels both inside and outside the workplace, were used to calculate the above statistical parameters for weekday measurements.

[a] 1995 values pertain to the original Bremen study performed by these authors during May 1995.

[b] 3-EP: 3-ethenylpyridine.

reported nonsmokers with a cotinine level above 15 ng ml^{-1} was 3%. However, it should be recognised that determined misclassification rates may be influenced by study design, for example, when comparing a random questioning and sampling protocol vs. participation in a study with financial incentives.

7. Results and discussion

7.1. Concentrations of ETS constituents to which Bremen subjects were exposed

In this publication, median values have been used as the primary means for reporting RSP and ETS marker concentrations since the data generated were highly skewed. These summary statistics, together with geometric means, arithmetic means, 10th percentile and 90th percentile values have been reported for each data set. For weekday measurements, time weighted average (TWA) particulate and vapour phase concentrations were determined for each individual subject, the calculations for which were based upon measured concentrations and the operational time over which the monitors were used inside and outside the workplace. Weekend monitoring was performed using a single personal monitor. Approximately 7.6% of all collected samples were excluded from the study, the flow rates for associated personal monitors found to be out of calibration on their return.

Comparisons were made between weekday and weekend monitoring during the winter and the summer for both 24-h and 7-day monitoring protocols in both smoking and nonsmoking locations. A comparison of 24-h weekday monitoring was also performed vs. the equivalent findings from our initial study performed in Bremen during 1995 (Phillips et al., 1998b). The significance of any concentration differences between data sets was examined by ranking each data set, with tied values receiving the average of the tied ranks, and comparing each data set with every other data set using Tukey's multiple range test. This was performed for RSP, SolPM, nicotine and cotinine only. Summary concentration data are presented in Tables 5–8 and relevant statistical comparisons are discussed in the text of this publication.

Throughout this publication, ETS concentrations, corresponding cigarette equivalent (CE) calculations and statistical comparisons between the various data sets have been

Table 7
Summary statistics for TWA particle and vapour phase concentrations determined for subjects performing 7-day sampling in smoking locations (Bremen)

Analyte	Collection period[a]	Number of subjects	10th Percentile	90th Percentile	Arithmetic mean	Geometric mean	Median
RSP (μg m^{-3})	Weekday winter	8	31	112	70	63	63
	Weekend winter	7	24	90	56	49	49
	Weekday summer	12	22	58	34	29	25
	Weekend summer	12	5.0	27	20	16	22
SolPM (μg m^{-3})	Weekday winter	8	10	88	49	36	46
	Weekend winter	7	2.3	52	25	8.1	19
	Weekday summer	12	0.29	27	8.9	1.6	0.99
	Weekend summer	12	0.05	2.3	0.66	0.16	0.05
FPM (μg m^{-3})	Weekday winter	8	14	89	49	39	42
	Weekend winter	7	6.6	58	30	18	24
	Weekday summer	12	4.6	44	15	9.5	6.3
	Weekend summer	12	0.36	9.1	4.4	2.1	3.6
UVPM (μg m^{-3})	Weekday winter	8	15	93	52	42	45
	Weekend winter	7	7.4	63	32	20	24
	Weekday summer	12	5.4	41	16	11	8.0
	Weekend summer	12	0.55	11	5.3	3.2	4.4
Nicotine (μg m^{-3})	Weekday winter	8	0.26	4.8	2.4	1.5	1.9
	Weekday winter	8	0.27	3.1	1.4	0.83	0.97
	Weekday summer	11	0.19	5.6	1.9	0.72	0.46
	Weekend summer	12	0.07	0.93	0.70	0.23	0.12
3-EP[b] (μg m^{-3})	Weekday winter	8	0.27	1.8	1.0	0.80	0.86
	Weekend winter	8	0.12	1.8	0.67	0.37	0.35
	Weekday summer	11	0.21	1.2	0.59	0.41	0.30
	Weekend summer	12	0.11	0.80	0.30	0.22	0.18

TWA exposure concentrations, determined for each subject from measured levels both inside and outside the workplace, were used to calculate the above statistical parameters for weekday measurements

[a] Weekday sampling performed over 5 days from Monday morning until work end on Friday. Weekend sampling performed from work end on Friday until Monday morning.

[b] 3-EP: 3-ethenylpyridine.

based upon SolPM and nicotine determinations. It has previously been suggested (Ogden et al., 1990) that SolPM determinations are more specific to ETS particles than the use of FPM or UVPM methodologies, which will be sensitive to sources of combustion other than cigarettes/ tobacco. Our observations support this, with reported UVPM and FPM concentrations being significantly higher than SolPM levels for low/no exposure groups. Reported 3-EP concentrations have not been used for subsequent exposure calculations due to the lack of mainstream data for this analyte, nicotine having been used in preference for ease of comparison with previous studies.

7.2. Respirable suspended particles

The highest median 24-h TWA RSP concentration (53 μg m^{-3}) was recorded for weekdays during the winter of 1999 for subjects both living and working in smoking environments. This value was significantly higher ($P<.001$) than the equivalent median RSP concentration determined during the summer (30 μg m^{-3}) but not significantly different ($P\geq.05$) from the value recorded during 1995 (39 μg m^{-3}). Equivalent median 24-h weekend concentrations were not significantly different from determined weekday levels during the winter, but were significantly lower (22 vs. 30 μg m^{-3}; $P<.05$) during the summer. With the exception of median 24-h weekend measurements made during the summer, all median 24-h (TWA for weekdays) RSP concentrations were significantly lower ($P<.01$) in nonsmoking locations, indicating a significant contribution to indoor RSP levels from tobacco smoke. A comparison of RSP and ETS particle concentrations for smoking and nonsmoking locations would suggest that the additional RSP associated with smoking locations could be entirely due to ETS particles. The sum of 24-h TWA ETS particle levels in smoking locations and corresponding RSP levels in nonsmoking locations were equivalent to corresponding RSP concentrations in smoking locations (53 vs. 53 μg m^{-3}, winter; 22.4 vs. 30 μg m^{-3}, summer). Median RSP levels determined for subjects performing monitoring over a full 7 days were not significantly different ($P\geq.05$) from equivalent weekday and weekend levels measured over 24-h periods. Figs. 1 and 2 depict the cumulative frequency distribution for TWA RSP and ETS particle (SolPM) concentrations in smoking and nonsmoking locations during the winter and summer, respectively.

7.3. ETS particles (SolPM)

The highest 24-h (TWA) median level for ETS particles (25 μg m^{-3}) was determined on weekdays for subjects both working and living in smoking locations during the winter of 1999, this concentration representing approximately 47% of measured RSP. This median concentration was one of the

Table 8
Summary statistics for TWA particle and vapour phase concentrations determined for subjects performing 7-day sampling in nonsmoking locations (Bremen)

Analyte	Collection period[a]	Number of subjects	10th Percentile	90th Percentile	Arithmetic mean	Geometric mean	Median
RSP ($\mu g\ m^{-3}$)	Weekday winter	9	18	42	30	28	28
	Weekend winter	12	22	41	31	30	32
	Weekday summer	10	15	25	20	19	19
	Weekend summer	10	7.6	16	13	12	14
SolPM ($\mu g\ m^{-3}$)	Weekday winter	9	0.06	25	9.4	1.5	1.1
	Weekend winter	12	0.05	8.0	2.4	0.47	0.64
	Weekday summer	10	0.05	0.70	0.33	0.10	0.05
	Weekend summer	10	0.05	0.05	0.05	0.05	0.05
FPM ($\mu g\ m^{-3}$)	Weekday winter	9	1.5	18	9.6	5.3	3.2
	Weekend winter	12	1.2	12	5.0	3.1	2.2
	Weekday summer	10	0.32	6.0	3.0	0.97	0.66
	Weekend summer	10	0.26	0.65	0.43	0.33	0.41
UVPM ($\mu g\ m^{-3}$)	Weekday winter	9	1.6	19	10	5.8	3.7
	Weekend winter	12	1.9	12	5.6	3.9	2.5
	Weekday summer	10	0.80	6.7	2.4	1.6	1.1
	Weekend summer	10	0.77	1.4	1.1	0.90	1.1
Nicotine ($\mu g\ m^{-3}$)	Weekday winter	9	0.02	0.60	0.25	0.10	0.11
	Weekend winter	10	0.01	0.92	0.31	0.07	0.03
	Weekday summer	10	0.01	0.15	0.07	0.04	0.03
	Weekend summer	8	0.01	0.01	0.01	0.01	0.01
3-EP[b] ($\mu g\ m^{-3}$)	Weekday winter	9	0.02	0.40	0.19	0.10	0.09
	Weekend winter	10	0.01	0.37	0.15	0.06	0.05
	Weekday summer	10	0.03	0.18	0.09	0.07	0.06
	Weekend summer	8	0.01	0.05	0.03	0.02	0.02

TWA exposure concentrations, determined for each subject from measured levels both inside and outside the workplace, were used to calculate the above statistical parameters for weekday measurements

[a] Weekday sampling performed over 5 days from Monday morning until work end on Friday. Weekend sampling performed from work end on Friday until Monday morning.

[b] 3-EP: 3-ethenylpyridine.

highest reported to date by these authors, approaching the median level determined for workers in the smoking workplace (8-h TWA, workplace only) of 37 $\mu g\ m^{-3}$ in Barcelona (Phillips et al., 1997a). This was an order of magnitude higher than the equivalent measurement made during the summer of 1999 (2.4 $\mu g\ m^{-3}$) and also significantly higher than equivalent 1995 levels (6.5 $\mu g\ m^{-3}$, $P<.001$). There was no significant difference ($P\geq.05$) between summer 1999 and 1995 weekday monitoring concentrations for smoking locations. The levels determined for 24-h weekend sampling in the winter were not significantly different from 24-h weekday measurements. However, equivalent summer levels were at least an order of magnitude lower for the weekend (0.12 $\mu g\ m^{-3}$ (<LOQ) vs. 2.4 $\mu g\ m^{-3}$; $P<.001$).

This large seasonal difference, particularly within smoking locations, may in part be explained by the temperate geographical region in which Bremen in situated. In regions where extremely high temperatures occur, the majority of offices and a large percentage of homes may be expected to have air conditioning systems, which for offices would almost certainly be operated to provide ventilation all year round. ETS concentrations in such work environments would therefore be expected to be more consistent over the course of a year. Offices where ventilation is likely to be provided via window openings during high temperature conditions, as may be expected for temperate locations, could be responsible for the large concentration differences observed between seasonal extremes.

As with RSP concentrations, all median 24-h (TWA for weekdays) ETS particle concentrations in nonsmoking locations were significantly lower ($P<.01$) than in smoking locations, with the exception of 24-h weekend measurements made during the summer. The highest median 24-h level for nonsmoking locations (1.9 $\mu g\ m^{-3}$) was recorded for weekdays during the winter of 1999, which surprisingly was not significantly different ($P\geq.05$) from the equivalent value reported for smoking locations during the summer of 1999. Median ETS particulate levels determined for subjects performing monitoring over a full 7 days were not significantly different ($P\geq.05$) from equivalent weekday and weekend levels measured over 24-h periods.

7.4. Nicotine

Reported nicotine data followed a similar pattern to that for ETS particles, although differences in concentration were not as pronounced for this vapour phase marker. The highest 24-h (TWA) median concentration was recorded for subjects performing weekday monitoring in smoking locations during the winter of 1999 (1.3 $\mu g\ m^{-3}$), which was significantly higher ($P<.05$) at five times the equivalent median concentration determined for the summer (0.26 $\mu g\ m^{-3}$). However, this concentration was not significantly

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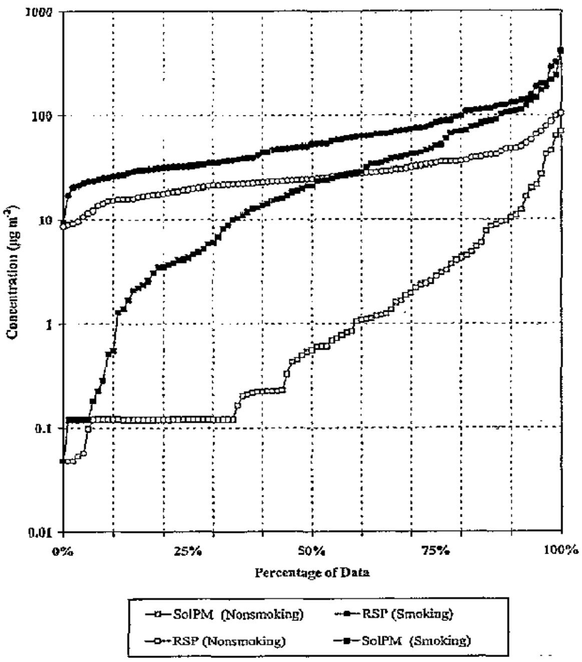

Fig. 1. Cumulative frequency distributions for TWA RSP and ETS particle concentrations determined during the winter (Bremen).

different from the equivalent value determined for the initial study performed in Bremen by these authors during 1995 (0.69 $\mu g\ m^{-3}$; $P \geq .05$). Weekend measurements in smoking locations were not significantly different from TWA weekday levels during the winter. Median levels were approximately an order of magnitude lower during the summer (0.03 $\mu g\ m^{-3}$ (<LOQ) vs. 0.26 $\mu g\ m^{-3}$; $P < .001$), again indicating a significant contribution from the workplace at that time of year. A recent review of studies investigating workplace exposures to ETS in the United States by Hammond (1999) summarised mean nicotine concentrations as generally falling between 2 and 6 $\mu g\ m^{-3}$ for offices and between 1 and 3 $\mu g\ m^{-3}$ for homes, where smoking takes place. This would position mean 24-h TWA weekday and 24-h weekend levels determined during the winter at the high end, and respective measurements made during the summer either below or at the very low end, of this scale. A similar range of mean nicotine concentrations (0.6–3.0 $\mu g\ m^{-3}$) was recently reported for fixed site monitoring in a number of smoking workplaces in Finland (Heloma et al., 2000).

In line with determined RSP and ETS particle concentrations, all median 24-h (TWA for weekdays) nicotine concentrations in nonsmoking locations were significantly lower ($P < .05$) than in equivalent smoking locations, with the exception of 24-h weekend measurements made during the summer. There was no significant difference between winter, summer or 1995 median 24-h TWA weekday concentrations determined for nonsmoking locations. Median 24-h weekend levels determined both in the summer and the winter were below the LOQ.

Median nicotine levels determined for subjects performing monitoring over 7-day periods were not significantly different ($P \geq .05$) from equivalent weekday and weekend levels measured over 24-h periods. Fig. 3 shows the cumulative frequency distribution for nicotine concentrations in smoking and nonsmoking locations for the summer and winter portions of the study.

7.5. Home and workplace exposures

The percent workplace contributions to median (TWA) RSP, ETS particle and vapour phase concentrations for all subjects are presented in Table 9. There were no appreciable differences apparent between workplace contributions during the winter and the summer, with values varying between 35% and 61% (with the exception of median SolPM levels in nonsmoking locations during the winter, of which 80% originated from the workplace). Since the workers investigated in Bremen spent, on average, one-third of their time at work during a weekday, concentrations were either comparable or greater in the workplace than outside the workplace based upon median levels.

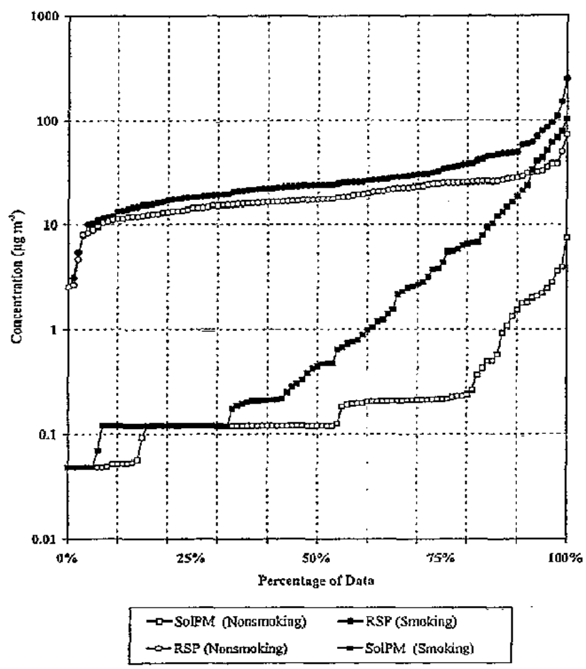

Fig. 2. Cumulative frequency distributions for TWA RSP and ETS particle concentrations determined during the summer (Bremen).

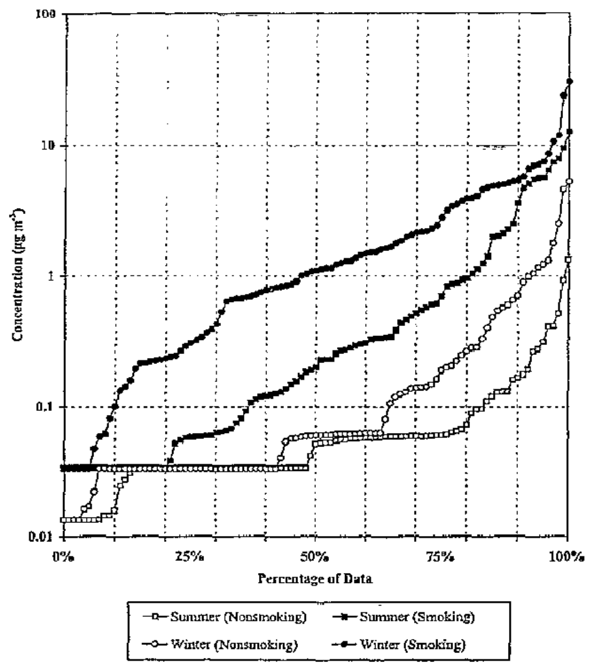

Fig. 3. Cumulative frequency distributions for TWA nicotine concentrations (Bremen).

7.6. Saliva cotinine and 3-hydroxycotinine

In this study, saliva cotinine and 3-hydroxycotinine concentrations were determined using a high-performance liquid chromatographic method with tandem mass spectrometric detection (Bentley et al., 1999), which had respective LOQs of 0.05 and 0.1 ng ml^{-1}. Using this method, less than 5% of cotinine data generated fell below the LOQ in either the winter or the summer portion of the study, compared with approximately 70% falling below the LOQ in the initial study performed in Bremen during May 1995. In comparison, a greater proportion of 3-hydroxycotinine data were below the LOQ, 31% and 48% for winter and summer, respectively, due to lower assay sensitivity and lower abundance for this analyte in saliva. In previous studies reported by these authors, a RIA method was used to determine saliva cotinine levels (Van Vunakis et al., 1987; Davis and Stiles, 1993) with between 44% and 79% of cotinine data generated falling below the reported LOQ of 1 ng ml^{-1}.

Saliva cotinine and 3-hydroxycotinine concentrations, expressed as an average of pre-, interim and postmonitoring levels, are reported in Table 10. Interim samples were not taken during the 1995 study, hence, reported saliva cotinine values are an average of determined pre- and postmonitoring levels only. There were no significant differences ($P \geq .05$) between subjects performing 24-h monitoring and 7-day monitoring in equivalent locations for either the winter or summer portions of the study. Fig. 4 shows the cumulative frequency distribution of average (mean of pre-, interim and post-air sampling measurements) saliva cotinine concentrations for subjects living and working in either smoking or nonsmoking locations for the summer and winter portions of the study.

For the largest group of subjects in this study, those performing monitoring over 24-h periods, highest median saliva cotinine levels were recorded for smoking locations during the winter portion of the study (1.2 ng ml^{-1}). This median concentration was significantly higher than the equivalent level determined for the summer study period (0.62 ng ml^{-1}; $P < .05$), but was not significantly different from the value reported for our 1995 study in Bremen (1.6 ng ml^{-1}; $P \geq .05$). Median levels determined in smoking locations were significantly higher than median levels determined for equivalent nonsmoking locations ($P < .05$). There were no significant differences ($P \geq .05$) apparent between any of the median levels determined for subjects both working and living in nonsmoking environments in the winter, summer and 1995 investigations.

Zuccaro et al. (1997), having investigated the interference of nicotine metabolites in cotinine determination using RIA, reported significant crossreactivity of 3-hydroxycotinine with anticotinine antiserum (34% at 50% inhibition). The antisera and reagents used for the determination of saliva cotinine in previous studies performed by these authors were the same as those investigated by Zuccaro et al., however, the extent to which this may have affected

Table 9
Median percent workplace contribution to TWA RSP, ETS particle and vapour phase concentrations determined for all subjects (Bremen)

Collection period	RSP	SolPM	UVPM	FPM	Nicotine	3-EP
Smoking locations						
24 h winter	42	47	50	46	61	51
7 days winter	36	36	37	35	44	40
24 h summer	42	50	51	56	47	46
7 days summer	41	58	44	44	39	47
Nonsmoking locations						
24 h winter	32	80	53	53	52	52
7 days winter	40	58	52	61	52	55
24 h summer	39	52	53	48	53	52
7 days Summer	36	47	44	52	49	46

3-EP: 3-ethenylpyridine.
Median values quoted in this table were derived from workplace contributions calculated for each individual subject using the following equation:

%Workplace contribution

$$= \frac{\text{Workplace sampling time}}{\text{Total sampling time}} \times \text{Workplace concentration} \times 100 / \left(\left(\frac{\text{Workplace sampling time}}{\text{Total sampling time}} \times \text{Workplace concentration} \right) + \left(\frac{\text{Home sampling time}}{\text{Total sampling time}} \times \text{Home concentration} \right) \right)$$

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Table 10
Summary statistics for saliva cotinine and 3-hydroxycotinine concentrations determined for all subjects (Bremen)

Collection period[a]	Number of subjects	10th Percentile	90th Percentile	Arithmetic mean	Geometric mean	Median
Cotinine (ng ml⁻¹)						
Smoking locations						
24 h winter	49	0.36	3.0	1.6	1.2	1.2
7 days winter	9	0.68	2.8	1.6	1.3	1.3
24 h summer	52	0.30	1.5	0.94	0.66	0.62
7 days summer	12	0.26	1.0	0.76	0.57	0.54
24 h 1995	18	0.50	3.1	2.1	1.5	1.6
Nonsmoking locations						
24 h winter	53	0.19	1.6	0.73	0.44	0.34
7 days winter	13	0.14	1.2	1.2	0.48	0.47
24 h summer	50	0.14	0.85	0.56	0.34	0.28
7 days summer	12	0.15	0.86	0.55	0.39	0.42
24 h 1995	36	0.50	0.88	0.66	0.61	0.50
3-Hydroxycotinine (ng ml⁻¹)						
Smoking locations						
24 h winter	49	0.13	1.1	0.51	0.37	0.38
7 days winter	9	0.27	0.99	0.62	0.49	0.51
24 h summer	52	0.07	0.75	0.36	0.20	0.15
7 days summer	12	0.09	0.29	0.19	0.17	0.16
Nonsmoking locations						
24 h winter	53	0.05	0.47	0.29	0.15	0.11
7 days winter	13	0.06	0.34	0.72	0.20	0.18
24 h summer	50	0.05	0.23	0.21	0.10	0.08
7 days summer	12	0.05	0.32	0.20	0.14	0.18

Values calculated using the average of pre-, interim and postmonitoring saliva cotinine or saliva 3-hydroxycotinine concentrations for each subject (1995 saliva cotinine values were calculated using the average of pre- and postmonitoring concentrations).

[a] 1995 values for saliva cotinine pertain to the original Bremen study performed by these authors during May 1995 (limit of quantitation 1.0 ng ml⁻¹).

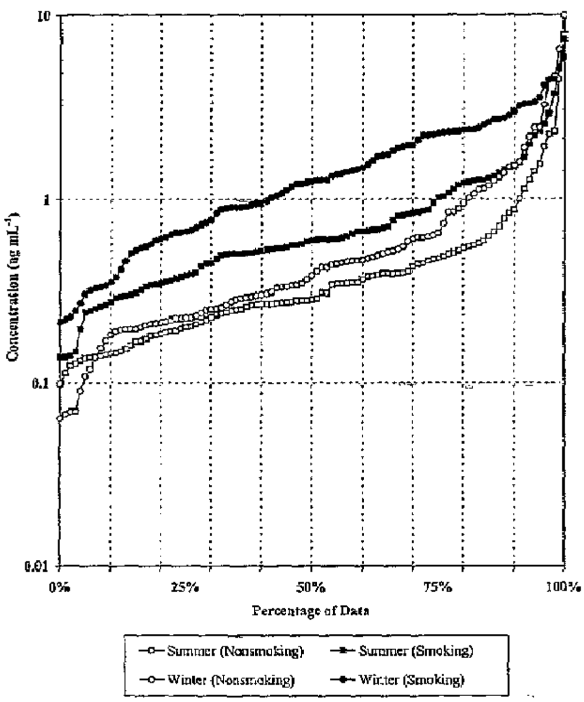

Fig. 4. Cumulative frequency distributions for average saliva cotinine concentrations (Bremen).

determined saliva cotinine concentrations is unclear. In a recent publication by Jenkins and Counts (1999), comparing data generated from the 16-city US study using RIA (Jenkins et al., 1996) with those generated as part of the Third National Health and Nutrition Examination Survey (NHANES III) (Pirkle et al., 1996), no evidence was found to support any such influence. It was surmised that any such impact would more likely be apparent for urine, where concentrations of 3-hydroxycotinine in relation to cotinine may be comparable or greater, whereas relative proportions in saliva appear to be much lower. In this investigation, cotinine and 3-hydroxycotinine concentrations were compared at 10th, 25th, 50th, 75th and 90th percentiles for each of the groups investigated during the summer and winter. Using data only from comparisons where both values were above the LOQ, the mean (±S.D.) proportion of 3-hydroxycotinine relative to cotinine in saliva was 34% (±6.9%). It is conceivable that cross-reactivity may have contributed to the fact that median nicotine levels were lower (0.69 vs. 1.3 $\mu g\ m^{-3}$) and saliva cotinine levels higher (1.6 vs. 1.2 ng ml⁻¹) during the initial study performed in 1995 than during the winter of 1999. However, these concentrations were not significantly different from one another.

Figs. 5 and 6 depict the correlation between saliva cotinine and airborne nicotine concentrations for subjects performing 24-h and 7-day monitoring, respectively. Here, 'interim' saliva cotinine levels vs. TWA weekday nicotine

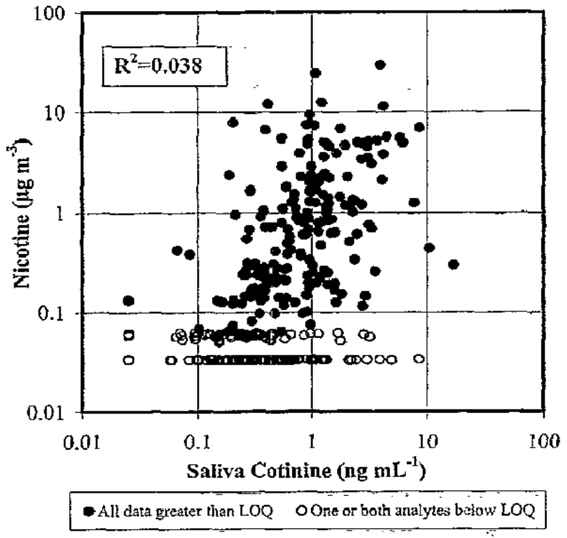

Fig. 5. Correlation of saliva cotinine with airborne nicotine for subjects performing 24-h sampling (Bremen).

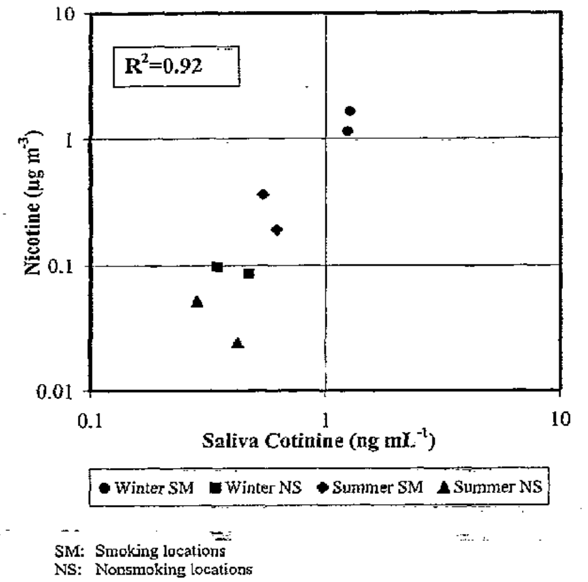

Fig. 7. Correlation of median saliva cotinine with median airborne nicotine levels for all subjects (Bremen).

concentrations and 'post' saliva cotinine levels vs. weekend nicotine concentrations have been plotted for each individual subject. The correlation between these parameters compared at the individual level was extremely poor for subjects performing 24-h monitoring and somewhat better, but still poor, for subjects performing 7-day monitoring (R^2 values of .038 and .21, respectively). In contrast, a very good correlation was apparent for a similar comparison made at

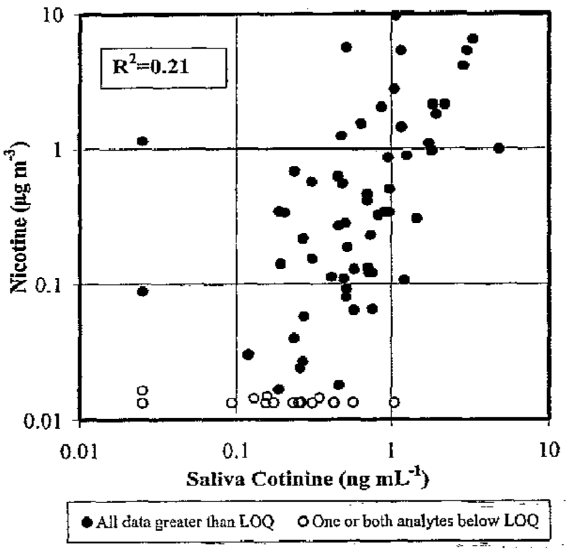

Fig. 6. Correlation of saliva cotinine with airborne nicotine for subjects performing 7-day sampling (Bremen).

group level. Fig. 7 shows the correlation between median saliva cotinine concentrations and median airborne nicotine concentrations (R^2=.92) for subjects in smoking and nonsmoking locations during the winter and the summer. Data from subjects performing 7-day monitoring and 24-h monitoring having been plotted separately, eight data points in total having been used for the regression analysis. Median saliva cotinine concentrations were calculated using the mean of pre-, interim and postsample levels for each subject and median nicotine levels were determined using calculated 7-day TWA nicotine levels using measured weekday and weekend concentrations, assuming a 5-day working week and a 2-day weekend.

Median saliva cotinine data from this investigation were consistent with determined median ETS particle and vapour phase concentrations. This would suggest that with the improved low level measurement, saliva cotinine has enhanced reliability as a biomarker for ETS exposure at group level.

7.7. Exposures to RSP, ETS particles and nicotine

The term "exposure" is often used when defining maximum allowable concentrations for hazardous compounds, and is normally determined by fixed site monitoring over standard time periods. In the context of this personal monitoring study, where concentrations cannot be directly related to a specific environment, "potential inhaled quantity" (PIQ) is used as a measure of overall exposure and was calculated as the product of the analyte concentration, the length of time

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subjected to such concentration and the breathing rate maintained throughout the period. A similar assumption was recently made by Ogden and Martin (1997) who noted that this provided "a more accurate accounting of total exposure among individuals as they engage in different activities in different microenvironments". Where PIQs have been quoted in terms of cigarette equivalents (CEs), these have been calculated in relation to the mainstream particle (tar) and nicotine yields of typical cigarettes. The values, 11 mg ETS particles and 0.8 mg nicotine, were calculated from the mean yields of the top six selling cigarette brand types in Germany. In this publication, CEs are used solely for conceptual comparison of exposures between groups of nonsmokers. The authors understand and accept that mainstream smoke inhaled by smokers has a different chemical composition to ETS, which comprises mainly aged and diluted sidestream smoke and the exhaled portion from smokers. The factor used to relate ETS exposure of nonsmokers with that for smokers, related to the prediction of potential health risks (Ogden and Martin 1997), was not applied.

Annual PIQs (mg), calculated for each subject group according to sampling period (24 h/7 day) and season (winter/summer) are summarised for smoking and nonsmoking locations in Tables 11 and 12, respectively. Annual

Table 11
Estimated annual potential inhaled quantities (PIQ) of RSP, ETS particles (SolPM) and nicotine for subjects in smoking locations (Bremen)

		Annual PIQ (mg)[b]			CEs	
Collection period[a]		RSP	SolPM	Nicotine	SolPM	Nicotine
Median levels						
Winter 1999	24 h	421	143	10	13	13
	7 days	453	306	12	28	15
Summer 1999	24 h	211	14	1.5	1.3	1.9
	7 days	181	7.1	4.6	0.65	5.7
1995	24 h	276	8.8	3.7	0.80	4.6
90th Percentile levels						
Winter 1999	24 h	913	738	48	67	60
	7 days	754	552	32	50	39
Summer 1999	24 h	630	246	17	22	22
	7 days	302	110	34	10	43
1995	24 h	704	269	17	24	22

[a] Winter and summer values represent annual PIQs calculated from determined winter and summer concentrations respectively, extrapolated to 1 year's exposure. 1995 values pertain to the original Bremen study performed by these authors during May 1995.

[b] Breathing rates of 0.65 $m^3 h^{-1}$ for females and 1.05 $m^3 h^{-1}$ for males were assumed. Annual PIQs for each individual were calculated as follows assuming a 48-week working year and that exposure concentrations during holiday periods were equivalent to determined weekend concentrations.

$$\text{Individual annual PIQ} = (\text{TWA 'weekday concentration} \times (0.65 \text{ or } 1.05) \times 48 \times 5 \times 24) + (\text{'weekend concentration} \times (0.65 \text{ or } 1.05) \times (365 - (48 \times 5)) \times 24)$$

During 1995, weekend sampling was not performed. ETS marker concentrations for workers at work and outside the workplace were calculated from the data provided by the 'work' and 'home' monitors. Accordingly, annual PIQs for each individual were calculated as follows assuming a 35-h working week and 48-week working year with the remainder of the time spent outside the workplace.

$$\text{Individual annual PIQ} = (\text{'work concentration} \times (0.65 \text{ or } 1.05) \times 48 \times 35) + (\text{'home concentration} \times (0.65 \text{ or } 1.05) \times ((365 \times 24) - (48 \times 35)))$$

Median and 90th percentile data were calculated for each cell from the individual PIQs as calculated.

Table 12
Estimated annual potential inhaled quantities (PIQ) of RSP, ETS particles (SolPM) and nicotine for subjects in nonsmoking locations (Bremen)

		Annual PIQ (mg)[b]			CEs	
Collection period[a]		RSP	SolPM	Nicotine	SolPM	Nicotine
Median levels						
Winter 1999	24 h	207	14	0.91	1.3	1.1
	7 days	203	26	0.64	2.4	0.79
Summer 1999	24 h	147	1.7	0.46	0.15	0.57
	7 days	120	0.46	0.16	0.04	0.20
1995	24 h	154	1.8	0.65	0.16	0.81
90th Percentile levels						
Winter 1999	24 h	358	81	5.0	7.4	6.2
	7 days	297	150	3.0	14	3.7
Summer 1999	24 h	239	10	1.3	0.92	1.6
	7 days	163	2.7	0.73	0.25	0.91
1995	24 h	302	9.7	2.3	0.88	2.9

[a] Winter and summer values represent annual PIQs calculated from determined winter and summer concentrations respectively, extrapolated to one year's exposure. 1995 values pertain to the original Bremen study performed by these authors during May 1995.

[b] Breathing rates of 0.65 $m^3 h^{-1}$ for females and 1.05 $m^3 h^{-1}$ for males were assumed. Annual PIQs for each individual were calculated as follows assuming a 48-week working year and that exposure concentrations during holiday periods were equivalent to determined weekend concentrations.

$$\text{Individual annual PIQ} = (\text{TWA 'weekday concentration} \times (0.65 \text{ or } 1.05) \times 48 \times 5 \times 24) + (\text{'weekend concentration} \times (0.65 \text{ or } 1.05) \times (365 - (48 \times 5)) \times 24)$$

During 1995, weekend sampling was not performed. ETS marker concentrations for workers at work and outside the workplace were calculated from the data provided by the 'work' and 'home' monitors. Accordingly, annual PIQs for each individual were calculated as follows assuming a 35-hour working week and 48-week working year with the remainder of the time spent outside the workplace.

$$\text{Individual annual PIQ} = (\text{'work concentration} \times (0.65 \text{ or } 1.05) \times 48 \times 35) + (\text{'home concentration} \times (0.65 \text{ or } 1.05) \times ((365 \times 24) - (48 \times 35)))$$

Median and 90th percentile data were calculated for each cell from the individual PIQs as calculated.

PIQs for ETS particles and nicotine in terms of CEs have also been reported. These have been calculated for each subject using the concentrations and sampling times determined from their individual weekday and weekend monitors and the assumed "awake" breathing rates of 0.65 m^3 h^{-1} for females and 1.05 m^3 h^{-1} for males (Holcomb 1993). Also assumed was a 48-week working year, with exposures during holiday periods being equivalent to determined weekend concentrations, and that no variation in ETS marker concentrations occurred throughout the year from those measured during the monitoring period. Median and 90th percentile PIQs were subsequently calculated for each subject group from these individually calculated daily exposures in order to represent "typical" and "highly exposed" subjects, respectively.

For comparison, annual exposures in terms of PIQs have also been reported for equivalent subjects from the study performed by these authors in Bremen during 1995. However, these exposures were calculated in a different way since only weekday monitoring was performed during this investigation. In this instance, annual PIQs were calculated for each subject from the data provided by the separate monitors worn in the workplace and away from the workplace. Subjects were assumed to spend 35 h/week and 48 weeks/year in the workplace, with no variation in ETS marker concentrations throughout the year, including weekends, from those measured during the monitoring period.

Based upon median levels of ETS particles and nicotine, highest exposures were received by subjects both living and working in smoking locations during the winter portion of the study. If annualised, by extrapolation of determined concentrations to 1 year's exposure, these levels would be equivalent to an exposure of between 13 and 28 CEs, significantly higher than equivalent annual exposures estimated using summer (0.65–5.7 CEs) or 1995 (0.80–4.6 CEs) measurements. Based upon 90th percentile values, the most highly exposed subjects from the winter portion of the study would be exposed to between 39 and 67 cigarette equivalents/year (CEs/year). These annual exposures estimated for the winter portion of the study are amongst the highest recorded for any of the cities investigated worldwide by these authors. The least exposed subjects, those living and working in nonsmoking locations, would receive less than 3 CEs/year based upon winter measurements and less than 1 CE/year based upon summer or 1995 measurements.

Although there were no significant differences apparent between equivalent measures for 24-h and 7-day monitoring groups, calculated annual exposures in terms of CEs showed greater variability between ETS particle estimates and nicotine estimates when applied to 7-day monitoring levels. This variability, which displayed no consistent trend vs. calculated CE estimates using determined 24-h levels, was most likely attributable to the low numbers of subjects recruited to perform 7-day monitoring.

8. Conclusions

Bremen was the thirteenth city investigated by these authors, assessing exposures to RSP and ETS in a randomly selected sample of the nonsmoking population. It was also the first city to be revisited to investigate seasonal exposure differences. The highest exposures to ETS particles and nicotine in this study were found during the winter portion of the study, performed during February/March 1999, for subjects both living and working in smoking locations. During this period, median levels for ETS particles were at least an order of magnitude higher and nicotine levels at least five times higher than the equivalent summer measurements. Extrapolating determined winter and summer levels to 1 year's exposure, these equate to potential inhaled quantities of between 13 and 28 CEs/year based upon winter measurements and between 0.65 and 5.7 CEs/year based upon summer measurements. These summer values were not appreciably different from those calculated using median exposures determined during our initial Bremen study in May 1995 of between 0.8 and 4.6 CEs/year. In all cases, determined levels for ETS particles and nicotine in smoking locations were significantly higher than in equivalent nonsmoking locations for both winter and summer portions of the study.

It was suspected prior to this investigation that there might be a seasonal influence upon the levels of RSP and ETS concentrations in indoor environments. The results from this investigation, however, have shown dramatic differences between levels determined during the winter and during the summer of 1999 in Bremen. Indeed, the differences were so marked that median levels determined in nonsmoking locations during the winter were not significantly different from those determined for smoking locations during the summer. In part, the temperate geographical region in which Bremen is situated may explain this large seasonal difference. Clearly, these findings highlight the need for careful consideration regarding the selection of monitoring periods and would indicate that, in certain circumstances, monitoring on a number of occasions may be required to more accurately represent annualised exposures to RSP and ETS. Further work to confirm these findings and to investigate seasonal variations in different geographical locations (e.g. equatorial wet/dry seasons) would be of benefit.

RSP, ETS particle and nicotine concentrations determined from subjects performing 7-day monitoring were not significantly different from the levels determined from those wearing personal monitors over two 24-h periods. However, calculated annual exposures in terms of CEs showed greater consistency between ETS particle estimates and nicotine estimates when applied to 24-h monitoring levels. The variability in annual exposure estimates derived from 7-day monitoring levels, which displayed no consistent trend vs. calculated CE estimates using determined 24-h levels, was most likely attributable to the low numbers of subjects recruited to perform 7-day monitoring. Although

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statistically there was no difference between the data generated over the two collection periods, the variability associated with 7-day monitoring warrants further investigation using a larger volunteer population, thereby enhancing the statistical power of the comparison.

In a recent review of occupational exposure to ETS and health risk, it was recommended that further research be undertaken to provide more accurate and precise estimates for the relationship between questionnaire-reported smoking activity and direct measures of ETS markers (Jaakola and Samet, 1999). This is particularly important since the majority of health effect studies base their ETS exposure assessment on questionnaires. This area of research could also be extended to provide a more direct relationship between ETS exposure and health effect by performing multioccasional personal monitoring of populations participating in long-term epidemiological investigations. Improvements in analytical methods, for example, to reduce the limit of quantitation for ETS markers, would also be of major benefit. The measurement of ETS air concentrations using solanesol as a marker has become more difficult in recent times due to lower levels of ETS in the atmosphere in general, most likely as a result of increased public awareness regarding ETS issues. By extending the monitoring period and developing an improved method for the determination of solanesol using LC–MS/MS, significant improvements in analytical specificity and achieved LOQ could be made. Consideration should also be given to other potential markers for ETS, including the use of heavy metals for the estimation of ETS particles. The use of cadmium for this purpose has shown great promise, as highlighted in a recent publication by Jenkins et al. (2000), although considerable method investigation would be required prior to any potential use as a routine tool for assessing ETS particle concentrations.

In this study, saliva cotinine levels were determined using a LC–MS/MS method capable of extremely low level quantitation (0.05 ng ml^{-1}), with the result that less than 5% of data generated fell below the LOQ. Reported levels determined using this technique also preclude any possibility of assay interference from associated nicotine metabolites. In previous studies performed by these authors, a RIA technique was used (LOQ 1.0 ng ml^{-1}), where typically 44–79% of saliva cotinine data were below the LOQ. The levels reported for this investigation were consistent with determined ETS particle and vapour phase concentrations and would suggest that low level saliva cotinine measurements have much improved reliability as a biomarker for ETS exposure when applied to subject populations.

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